U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-279 151

Guidelines for Zooplankton Sampling in Quantitative Baseline and Monitoring Programs

Virginia Inst of Marine Science, Gloucester Point

Prepared for

Corvallis Environmental Research Lab, Oreg

Feb 78

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/3-78-026		PB279151

4. TITLE AND SUBTITLE	5. REPORT DATE
GUIDELINES FOR ZOOPLANKTON SAMPLING IN QUANTITATIVE BASELINE AND MONITORING PROGRAMS	February 1978
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Fred Jacobs and George C. Grant	Special Scientific Report No. 83
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Department of Planktology Virginia Institute of Marine Science Gloucester Point, VA 23062	1 BA 025
	11. CONTRACT/GRANT NO.
	EPA-R-804147010
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Corvallis Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Corvallis, Oregon 97330	Final Report
	14. SPONSORING AGENCY CODE
	EPA/600/02

15. SUPPLEMENTARY NOTES

16. ABSTRACT

Methods applicable to zooplankton sampling and analysis in quantitative baseline and monitoring surveys are evaluated and summarized. Specific recommendations by managers must take into account characteristics of the water mass under investigation, the abundance of contained zooplankton and phytoplankton populations and the objectives of the study. Realistic planning and development must also consider available monetary and manpower resources.

This report was submitted in fulfillment of Contract No. R804147010 by the Virginia Institute of Marine Science under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from 24 Nov 75, to 31 May 77, and work was completed as of 25 Feb 77.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Marine biology Sampling Water pollution Zooplankton		08/A

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to public	unclassified	62
	20. SECURITY CLASS (This page)	22. PRICE
	unclassified	A04, A01

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

☆ U.S. GOVERNMENT PRINTING OFFICE: 1978—799-946/86 REGION 10

PB 279 151

EPA-600/3-78-026
February 1978

Ecological Research Series

GUIDELINES FOR ZOOPLANKTON SAMPLING IN QUANTITATIVE BASELINE AND MONITORING PROGRAMS

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Environmental Research Laboratory
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EPA-600/3-78-026
February 1978

GUIDELINES FOR ZOOPLANKTON SAMPLING IN QUANTITATIVE BASELINE AND MONITORING PROGRAMS[1/]

by

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Grant Number -
EPA-R804147010

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CORVALLIS, OREGON 97330

1/ Special Scientific Report No. 83, Virginia Institute of Marine Science

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FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine ecosystems; the behavior, effects and control of pollutants in lake systems; and the development of predictive models on the movement of pollutants in the biosphere.

This report presents a review of methods for sampling and analyzing marine zooplankton communities. These quantitative techniques can be used to establish ecological baselines or to conduct surveys of the impact of pollution on zooplankton dynamics.

A. F. Bartsch
Director, CERL

ABSTRACT

Methods applicable to zooplankton sampling and analysis in quantitative baseline and monitoring surveys are evaluated and summarized. Specific recommendations by managers must take into account characteristics of the water mass under investigation, the abundance of contained zooplankton and phytoplankton populations and the objectives of the study. Realistic planning and development must also consider available monetary and manpower resources.

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ACKNOWLEDGMENTS

The authors wish to thank Dr. Richard C. Swartz of the Environmental Protection Agency for his helpful suggestions in developing this report and for critically reviewing the final draft. We also thank Mrs. Shirley Sterling for her very able secretarial assistance.

SECTION 1

CONCLUSIONS

The zooplankton is a diverse assemblage of animal forms, and as a result, exhibits wide spatial, diurnal and seasonal variations in abundance and composition. Accurate interpretation of quantitative and qualitative relationships are further complicated by gear bias and the ability of certain groups to avoid capture. Methods to minimize error estimates follow no standard procedure; only careful analysis of the problem under study facilitates the proper approach.

Baseline studies are required to be broader in scope and more detailed in their enactment than are monitoring studies. Frequency of sampling, number of stations, accumulation of hydrographic information, etc., should be expansive for baseline studies. Monitoring should be initiated when phenomena are somewhat understood and/or patterns from previous baseline studies have been developed. As a result, stations in monitoring studies are usually set further apart than in original baseline studies. However, characteristics of the water column being sampled are most important in determining the location of sampling sites. Sampling locations might be further apart in homogeneous waters, such as certain offshore areas, than in heterogeneous coastal waters. In estuaries, where environmental parameters exhibit considerable variations over relatively short distances and time periods, a closer spacing of sampling sites and an increased frequency of sampling are recommended. Sampling sites are often randomly selected stratified stations chosen from a gridded pattern which has been overlaid on the study area. Transects may be utilized in situations where the study area covers great distances and ship time is limited. In pollution studies, a series of transect lines radiating from a source point may be advisable. Furthermore, stations along these transects should extend into unaffected areas. In studies concerned with small scale distribution of zooplankton, a parachute drogue can be employed to maintain station locations for repeated sampling from the same water parcel.

Pumping systems, although expensive, are most efficient for capturing microzooplankton, but these systems should pump in excess of 150 l/min. to minimize avoidance.

Nets are recommended for sampling mesozooplankton. There is no ideal plankton net; appropriate gear selection is dictated by a clear understanding of the problem being studied. The mouth opening of the net should be as large as possible, and still retain its ability to be handled efficiently aboard ship. Nets with mouth openings of 50 to 100 cm in diameter (or 0.2 m^2 or greater) are adequate for capturing most groups. Tow speed should be

between 1 1/2 - 2 knots for low speed nets, and the net should maintain a constant velocity while under tow. Mesh size selection is critical in determining quality and quantity of the catch. In relatively plankton rich, temperate coastal waters, 333 µm is the minimum mesh size that maintains a filtration efficiency of 85% or greater. Although a 202 µm mesh net has the advantage of retaining forms that pass through a 333 µm mesh net, filtration efficiency drops after 5 minutes of tow in these waters. In areas of low plankton biomass, where clogging is not likely to occur, the 202 µm net is recommended. All nets should be properly cared for, kept out of direct sunlight, and washed in fresh water at the conclusion of each cruise.

Samples should be properly labelled and generally are best preserved in 4% buffered formaldehyde. Sampling zooplankton for pollutants requires special methodology, largely designed to minimize potential contamination. Splitting of samples is recommended for quantitative studies; two types of splitters are suggested, the Folsom & the Burrell. Zooplankton groups should be split to workable numbers (usually 100-200 individuals within a group).

Paired nets provide a replicate sample that can be utilized for biomass considerations. The most popular techniques for presenting biomass are: (1) Settling volume, (2) Displacement volumes, (3) Wet weight, (4) Dry weight and (5) Ash-free dry weight. Volume measurements and wet weight are non-destructive measurements. Information on the nutritional content of the zooplankton is provided by dry weight and ash-free dry weight. Dry weight is best determined by freeze-drying samples.

The patchy distribution of zooplankton in the marine environment dictates care in the application of appropriate statistical methods of analysis. Observations made from ranking, abundances of individual groups, species and biomass, still form the bulk of the accumulated data from baseline and monitoring surveys. Spatial and seasonal trends can be interpreted in this manner. Diversity indices can be used for zooplankton community comparisons and pollution studies. These indices may have added value when used in conjunction with other indices, associated statistics and observations. With the widespread availability of computers, multivariate approaches are receiving greater attention. This type of analysis draws its conclusions by recognizing patterns among variables and by condensing multidimensional relationships. By using clustering techniques, samples may be partitioned, distinguished and ranked, according to similarities of species composition and abundance.

SECTION 2

INTRODUCTION

2.1 Zooplankton

The term zooplankton refers to individuals and communities of animals, whose distribution and dispersal are influenced significantly by the movements of the waters. Zooplankton are essential intermediary links in marine food chains since they graze on phytoplankton, and provide a direct food source for more complex animal forms. Furthermore, the zooplankton impart excretion products and organic detritus to the marine environment, and planktonic organisms constitute a considerable portion of the biomass of the world's oceans. Almost every animal phylum is represented, at least at some life stage, in the plankton.

This great diversity of animal types makes the zooplankton a difficult community to work with. Specialists are required for individual groups and quantitative sampling is beset with problems. In addition to errors associated with present sampling methodology, a greater source of sampling difficulty is the "aggregate" or "patchy" nature of the zooplankton itself.

2.2 Spatial variations

In the late 1800's and early 1900's opposing opinions concerning the distribution of oceanic plankton were held. Despite Haeckel's (1890) efforts to persuade the scientific community that, "the composition of the plankton is in qualitative as well as quantitative relations, very irregular," Victor Hensen, working with Kiel planktologists, firmly advocated relatively even distribution. Hensen's theory implied that plankton data obtained from a few hauls, could be used to estimate conditions over a much greater area of the ocean. Haeckel's hypothesis implied that individual areas of the sea had to be sampled before anything could be stated about plankton communities within these areas. The controversy was ended by results of A. C. Hardy's (1936) "Discovery" and "William Scoresby" expeditions. By utilizing a continuous plankton recorder, which sampled plankton densities and fluctuations along continuous lines of observation, Hardy concluded that "there is no doubt as to the patchy nature of the oceanic plankton."

Explanations offered for the existing patchy distribution of oceanic plankton are numerous, ranging in physical, chemical and biological factors. Selected references dealing with this subject are presented in the bibliography on this section. These aggregates of zooplankton vary in size and shape, but several researchers have indicated patch diameter to be hundreds of meters

or less. Densities within patches are generally from 2 to 5 times greater than background densities. Results from computer simulated models and actual field studies have indicated that the size and distribution of patches in a body of water greatly affect the accuracy and precision of estimates of zooplankton abundance.

2.3 Temporal variations

Natural fluctuations associated with seasonal variations in sunlight intensity, water temperature and transparency, oxygen and nutrient content of the water etc. exert direct and indirect effects on zooplankton survival and community development. As a result, wide ranges in zooplankton biomass and specific abundance occur. At a single station in the Sargasso Sea, variations in biomass changed by a factor of 10, between certain seasons (Deevey 1971). In Chesapeake Bay, biomass in summer months can be two orders of magnitude greater than in relatively sparse periods; differences in actual numbers of individuals within groups can vary by as much as 5 or 6 orders of magnitude between seasons (Jacobs and Grant MS.). Furthermore the change of dominant species, over time, results in distinctive zooplankton communities during certain periods of the year. The degree to which naturally occurring seasonal phenomena affects variability in zooplankton numbers and species must be considered when designing a zooplankton study.

Many zooplanktonic organisms perform diurnal vertical migrations, the effects of which are most pronounced in the upper 300 m. An early model indicated that upward migration begins at noon, and organisms attain their highest water column levels near midnight. The effects of light and the physiology of the organisms themselves appear to be the most important controlling factors in diurnal migration. The likelihood of sampling error is further increased during darkness since migrating species tend to be compressed into more discrete layers during night hours, than are nonmigrating species. An important consequence of vertical migration is that no two samples from the same body of water, unless taken at the same time of day, are directly comparable. In "Plankton and Productivity of the Oceans", Raymont (1963) devotes a chapter to vertical distribution of zooplankton and reviews the early literature on diurnal migration.

There appears to be, at least in nearshore waters, a periodic fluctuation in zooplankton abundance and composition that is directly related to tidal condition. This is generally more prominent in species that do not undergo diel vertical migrations (Sameoto 1975).

2.4 Introduction to bias and variation associated with sampling

2.4.1 Introduction to sampling gear bias

In addition to variations related to the plankton and its environment, additional error and bias is introduced by the choice of gear, and the manner in which it is used. Proper gear selection is essential in attempting to maximize accuracy, but no "blanket" recommendations for the "ideal" gear types can be offered. Only careful analysis of the problem under study will

facilitate the correct choice(s). Naturally, factors such as ship time and capability, funds for study, and available manpower are, practically speaking, as important as the questions under study, for selecting gear. With many samplers, variables such as the speed, depth and duration of tows can affect resulting catches. When nets are used, different mouth openings, mesh sizes, netting material and net structure influence the biomass and constituency of the captured organisms. This report will present a variety of sampling situations and offer appropriate suggestions and recommendations to maximize sampling efficiency.

2.4.2 Avoidance of samplers by zooplankton

Although discussed in greater detail later in this report, the subject of avoidance deserves brief mention here. Error and variation in estimating zooplankton is increased by the ability of certain groups to avoid capture while others cannot. This results in selective sampling and in general underestimation of zooplankton abundance and biomass. Mechanisms of avoidance are numerous: responses to changes in water movements, size, swimming ability, etc. of the animals are all factors. Certain age or physiologically stronger groups within a species may exhibit a greater ability to avoid capture than others. Naturally, avoidance is greatly influenced by the type(s) of gear utilized, and the manner in which it is used. For further information, a UNESCO report that summarizes the exhaustive literature on avoidance, is recommended (Clutter and Anraku 1968).

SECTION 3

SAMPLING

3.1 Sampling site selection and station selection

The problem under study, time, and available money, are essential considerations for sampling site selection. The type of information required dictates the scientific approach. For example, baseline and monitoring studies, although related, have distinct differences in the scope of information they seek, and therefore require different thought processes in their development. Subsequently, the location, frequency, and number of stations sampled should be quite comprehensive for initial baseline studies. Baseline studies should also include intensive sampling of a wide variety of related measurements, such as dissolved oxygen, salinity, temperature and other aspects of water chemistry (i.e. nutrients). Selection of sampling sites can be facilitated by dividing the area into a gridded pattern or into transects. Once data and information are analyzed, phenomena are better understood and trends established, scaled down monitoring can be initiated. Numbers of stations and samples can be reduced, if it is feasible to do so without information loss. Essentially, monitoring programs should be designed to provide maximum information for minimum cost and effort.

The design of the sampling program must consider both the objectives of the investigations and the hydrographic conditions in the area of study. Sampling locations might be further apart in offshore areas of homogeneous water characteristics, than in rather heterogeneous neritic areas, where close sampling sites might be indicated. In estuaries, where environmental variables exhibit considerable variations over relatively short distances, an even closer spacing of sampling sites is recommended.

In oceanic studies, the sampling patterns may consist of a series of transects across different current systems, a series from shore to deeper waters, or a grid of nearly equidistant stations within an oceanographic area. Although gridded mapping of a study area is most desirable, this is not always possible in studies that monitor large distances and have fixed time requirements. When a grid pattern is utilized, it is usually overlaid on a study area that has been divided into subareas. These subareas are generally determined by hydrographic factors. Within each subarea, random stratified stations may be selected. It is advisable to draw up a cruise track having a clear indication of the projected ship time, both available and required, to complete the station program. It is recommended that additional alternate stations are included in the sampling program. Furthermore, researchers should pre-determine which stations are "least crucial" to the study, in the event that time factors dictate elimination of certain stations.

Frequency of sampling is also dependent on the specific objectives to be answered and available ship time. Generally, ship requirements differ for different studies; estuarine areas can, as a result, be more frequently sampled than offshore waters. It is desirable and feasible to conduct surveys in estuarine waters monthly or biweekly, while offshore monitoring is usually conducted bi-monthly, quarterly or semi-annually. All available published literature and hydrographic data accumulated for an area of potential study should be utilized to maximize efficiency of sampling design.

In cases of pollution related monitoring problems, it is preferable that a series of samples should extend from affected to unaffected areas, the latter samples then serving as controls. If this is not possible, control data can be provided from surveys in the same area, conducted previous to the time of the introduction of a pollutant, or from other unpolluted areas of similar hydrographic and environmental structure. Sampling sites should be close enough to show gradient effects when they exist. Monitoring should continue until recovery is complete. In studies that monitor effects from a direct environmental addition, stations can be established along a series of transect lines, radiating from a source point.

For sampling a specific water parcel, the use of a parachute drogue, set below the surface, provides a suitable reference point for navigation and station location. Relative positions of station sites are maintained, since modifications in the sampling locations are determined by movements of the drogue. In this way the same parcel of water can be repeatedly sampled, regardless of the influence of the currents. This is particularly useful in field studies that are concerned with small scale variations in zooplankton.

3.2 Sampling gear

Three general classes of sampling gear are used to sample zooplankton, namely water bottles, pumps, and nets. This section will devote a major emphasis on net samplers, since they are most efficient for capturing organisms larger than micro-zooplankton.

3.2.1 Water bottles

The use of water bottles provides samples and associated hydrographic data from fixed locations within the water column. For most purposes 10 liter bottles are recommended. These samplers can capture and return live organisms; however, animals capable of only moderate locomotion can avoid capture. Water bottles, widely used in phytoplankton sampling, are of value only in sampling microzooplankton (animal plankters that pass through a 202 μm mesh net).

3.2.2 Pumping systems

This is probably the most favorable method for capturing microzooplankton. These systems generally control filtering aboard ship, with water being run through a single (or a nest of different sized) filter(s). Samples are then preserved and generally analyzed by direct counts with an inverted microscope. Pumping systems can be towed from moving vessels and can provide integrated samples over a range of depth. The depth range capability is a function of

the amount of hose attached to the pump. To minimize avoidance these systems should pump in excess of 150 l/min. If the capture of larger forms (i.e. copepods) is desired, pumping rate should be in excess of 200 l/min. In certain cases, pumping may cause partial or total destruction of soft bodied forms. Another disadvantage of pumping systems is that they tend to be expensive and require specific shipboard modifications.

3.2.3 Introduction to net sampling

Although this is the best method for capturing larger forms, sampling microzooplankton with nets is not recommended. To retain microzooplankton, net meshes must be small and as a result, samplers tend to clog rapidly. A 103 µm mesh has been demonstrated to fall below 85% filtering efficiency during the first minute of tow (Smith et al. 1968).

The grouping of zooplankton by size classes generally follows this scheme:

(1) microzooplankton - defined as zooplankton that pass through a mesh size of 202 µm;

(2) small mesozooplankton - defined as zooplankton retained by a mesh size of 202 µm;

(3) large mesozooplankton - defined as zooplankton retained by 1 mm mesh size;

(4) macrozooplankton - defined as large agile plankters and less agile nekton (captured by such gear as an Isaacs-Kidd Midwater Trawl).

Although there is no "ideal" zooplankton net available, attempts should be made to limit choices for given situations. This would improve standardization and result in greater comparability of different studies. Researchers, however must have a clear definition of the problem under study; this generally facilitates the appropriate choice of gear. For example, Vannucci (1968) pointed out the differences in approach when sampling for fish larvae and mixed plankton. "The fisheries manager tries to select certain size classes and or species against the others, while the planktologist tries to obtain as representative a sample as possible of the mixed association living in the sea." A 505 µm mesh net may be adequate for sampling fish larvae, but would be of limited scientific value for sampling immature copepods and other smaller forms.

3.2.4 General considerations of net sampling

(1) Mouth opening - nets with different sized mouth openings have been shown to have selective capabilities for the capture of zooplankton species. The importance of mouth opening for the efficiency of capture has been examined in actual field and computer simulated models. It appears that 20 and 40 cm nets introduce error by underestimating abundance and diversity (McGowan and Fraundorf 1966; Wiebe and Holland 1968). Larger mouth openings (100 cm or greater) yield more precise data and capture larger forms. However the larger the net, the more difficult it is to handle and retrieve. Although larger nets are more desirable, nets with mouth openings of 60 cm diameter (or 0.2 m^2) are considered adequate for ocean sampling. When sampling for macrozooplankton, little difference

was noted between 6 and 10 foot Isaacs-Kidd midwater trawls for most groups, excluding the fishes (Friedl 1971). The 10 ft trawl captured significantly more numbers and individual species of fish, than did the 6 foot trawl.

(2) Tow speed - This can be an important factor, considering the avoidance capabilities of certain species. Tested species of copepods were able to avoid nets towed at 30 cm/sec (0.6 knots). It is therefore recommended that low speed nets be towed between 1 1/2 and 2 knots (75-100 cm/sec). Even at constant engine speeds (between 1 and 2 knots), variation in net speed through the water sometimes exists, and may introduce significant error in the interpretation of data.

High speed samplers (>3 knots) have been developed for use from moving vessels. Although records indicate that these samplers sometimes damage organisms, other reports found these samplers to capture and return intact organisms. Reduction of mouth opening size avoids conditions of clogging in these nets. At speeds of 1 1/2-2 knots most plankton are captured; high speed should be required only for sampling specific groups (primarily fishes) capable of considerable movement.

(3) Mesh size - In temperate, relatively plankton rich coastal waters, it has been demonstrated that 333 µm is the minimum net mesh size that maintains over 85% filtration efficiency for the duration of 15 minute tows (Smith et al. 1968). Smaller sizes tend to have reduced filtration efficiency due to clogging. Filtration efficiency is calculated by comparing results of two flow-meters, one mounted inside the net mouth opening, the other outside,

$$\text{Filtration Efficiency (\%)} = \frac{\text{observed volume of water through net (from inside flowmeter)}}{\text{theoretical maximum volume of water through net (from outside flowmeter)}} \times 100$$

Since the 202 µm mesh net delineates microzooplankton from small mesozooplankton, it would appear logical that this net receive wide usage, and it has. A UNESCO (1968) report recommended using a 200 µm mesh net for sampling small mesozooplankton. Unfortunately, this net tends to clog in plankton rich waters, filtration efficiency dropping below 85% in 5 minutes. It does, however, have the advantage of retaining forms that pass through 333 µm mesh net. Whether this would add significant information to a problem under study, must be considered by individual investigators. In areas where clogging is not likely to occur, this mesh size (202 µm) is recommended.

(4) All nets should be made of nylon material.

(5) When nets are under tow, wire angle should be 30° or less. Nets should not be raised faster than 45 meters/min during oblique or vertical tows.

(6) Other factors such as filtration to mouth area ratios, bridles, color of nets, extrusion through meshes, etc. can all affect the net's efficiency.

3.2.5 Recommended nets for oceanic sampling

Of the dozens of samplers available (see Jossi, 1970) only three types are discussed below:

(1) Multiple opening-closing nets - These systems are now becoming popular at certain major oceanographic laboratories. This system provides information on intermediate (100's to 1000's of meters) scale spatial patterns of zooplankton distribution and can be equipped with sensors that monitor related environmental parameters. The sampler contains several nets, each of which is opened and closed sequentially by commands through conducting cable from the surface (Wiebe et al. 1976). This system provides the best technology developed to date for studying the vertical distribution and profiles of marine zooplankton species. This system is, alas, quite expensive and large; its use is limited to well equipped, large ocean research vessels.

(2) Paired opening-closing Bongo system - This sampler consists of two mechanically operated opening-closing nets mounted side by side (McGowan and Brown, 1966). These nets are recommended over single net samplers, in that they provide two samples from the same environment. One can be used for taxonomic purposes, while biochemical analyses can be conducted on the other. The mouth opening of each net should be at least 50 cm in diameter (60 cm is more desirable) and have a mesh area to mouth opening area ratio of at least 5 to 1. Used concomitantly with a pinger and/or a Time-Depth Recorder, this system yields an integrated sample over a desired depth range. Digital flowmeters should always be used to monitor water flow. This system is much less expensive than multiple opening-closing nets and is easier to handle. Although multiple opening-closing nets are preferable for studying detailed vertical structure, paired opening closing systems are adequate when single integrated samples over a depth range are required.

(3) Longhurst-Hardy Plankton Recorder - This sampler has been developed for studying small scale patterns (10's to 100's of meters) of spatial zooplankton distribution. This net utilizes the Hardy principle, of capturing plankton on gauze in the cod end. The gauze is advanced at periodic intervals, and can be changed by spooling. This sampler exhibits a certain amount of bias, probably in part due to its relatively low mesh aperture area to mouth opening area ratio (stalling and other residence time biasing of organisms sometimes being significant). Recently, Haury et al. (1976) have conducted several experiments on the sources and degree of bias associated with Longhurst-Hardy Plankton Recorders. Recommendations are made for proper design and use of this sampler.

3.2.6 Care of nets

(1) After sample is removed from net, by carefully hosing organisms into cod end, net is rinsed without a cod end.

(2) If clogging is severe, nets should be washed in detergent.

(3) Net should never be left in direct sunlight for extended time periods.

(4) Both net and flowmeter should be washed in fresh water, after each cruise (flowmeters washed more often during cruise, if possible).

SECTION 4

SHIPBOARD HANDLING OF SAMPLES

Most of the recommendations from this section are taken directly from Griffiths et al. (1976). Samplers should always be handled carefully in a manner to minimize injury to zooplankters and scientists.

4.1 Labelling and shipboard handling of samples

Glass jars with screw on plastic lids are generally used for storing zooplankton. Jars should be labelled on the outside with pre-printed gummed labels, this can be done in the laboratory prior to ship time. Labels should never be placed on jar caps. A sample label used by the Virginia Institute of Marine Science (VIMS) Planktology Department is presented below:

VIMS PLANKTOLOGY DEPT.

SHIP _____ CRUISE NO. _____ COLL. NO. _____

DATE _____ GEAR _____ MESH SIZE _____

STA. NO. or LOCATION _____

In addition to outside labels, an internal label written on water-resistant paper should be placed in every jar. Black India ink is preferred over pencil marks because it provides a permanent, waterproof, easy-to-read record. Internal labels should also be pre-printed.

Collecting information should be contained in a field log as the samples are being collected. A sample page from a VIMS field log is presented on the following page. Generally, comments on sea state, hydrography and water chemistry are kept on a separate form(s).

4.2 Preservation of samples

Formaldehyde is commonly used as a fixative for preserving zooplankton samples, a saturated solution containing 38-40% formaldehyde is known as, "concentrated formalin". One part formalin should be added to nine parts sea water, to provide a 4% formaldehyde solution. Buffering of concentrated formaldehyde should be conducted by adding 2 gms. of borax to 98 ml of

BLM Bongo Collections

Station No. _____ Collection No. _____

Location (start) _____ Depth _____

Location (end) _____

Date _____ Vessel _____

Time (EST) opened _____ Meter final rdg. _____

Time at depth _____ " initial rdg. _____

Max fishing depth _____ No. revs. _____

Time closed _____ Meter No. _____

Net size and mesh _____ Vessel speed _____

Type of tow (stepped or continuous oblique) _____

Depressor used _____ Wire angle _____ Wire out _____
(at max. fishing depth)

Remarks:

concentrated formalin, before dilution. Periodically, the neutrality of the sample should be checked with pH paper. Calcareous animals should be preserved in formalin with a pH of 8.2, since calcium carbonate dissolves below that level. The use of instruments that can dispense formalin is recommended. Furthermore, formaldehyde can be very harmful; it should be washed off skin immediately. Splashes in eyes should be followed by washing in cold water for 10-15 minutes, and medical attention.

4.3 Shipboard procedures for obtaining samples

(1) Secure flowmeter(s) propeller (use of TSK or GO type meters are recommended). Read and record number of revolutions obtained during tow.

(2) Wash net from the outside, starting at the mouth and working downward toward the cod end. Use a sea water hose whenever possible.

(3) Remove the bucket or bag from the cod end; concentrate sample through a concentrating device of mesh size equal to or smaller than the mesh of the net. This must be done quickly to minimize damage.

(4) Plankton should be washed into the labelled sample jar, filled 3/4 full with sea water. The volume of the plankton should not exceed 10% of the jar. If catches are larger, plankton should be placed in a larger jar or divided into 2 jars. If divided, the jars should indicate either, "1 of 2 samples" or "2 of 2 samples" etc.

(5) Add formaldehyde, preferably from a dispenser. Final concentration should be 4%. Gently rotate jar.

(6) Place inside label in jar, seal cap tightly and invert the jar several times periodically during the first hour of fixation.

(7) Before proceeding to next station, check to see that data have not been omitted from field logs.

(8) Store the jars in a cool, dark, place.

(9) Upon return to the laboratory, the state of the sample preservation and sample pH should be periodically checked.

(10) Shipboard procedures for obtaining biomass samples are discussed in section 5.3.

4.4 Special cases

4.4.1 Sampling for pollutants

Problems arise when sampling zooplankton for pollutants, such as trace metals, pesticides or petroleum hydrocarbons. Since these levels are extremely low in the natural environment, and ocean-going vessels are a generous source of these materials, the potential for obtaining contaminated specimens is great.

Certain precautions can, however, be taken to minimize contamination:

(1) Specimens should never have any contact with the surface of the ship.
(2) Contact between collecting gear and ship surfaces should be minimal.
(3) Neither sample nor gear should be washed with the ship's salt water.

(4) Hands, tools, sorting trays and storage containers should be liberally washed with 95% ethanol.

(5) Handling and sorting gear must be of metal, glass or enamel.
(6) Use metal cups for pesticide collections.

(7) Use of opening-closing nets can be used to avoid contamination from the ship (i.e. paint chips). Sampler is opened and closed below the surface.

4.4.2 Sampling gelatinous organisms

Sampling gelatinous zooplankton with nets may prove to be inadequate for certain groups. Many of these organisms are delicate and are lost or damaged beyond recognition when strained through meshes. Furthermore, many of these groups do not preserve well in formaldehyde and require specialized methodology for fixation and preservation. Current information on biology and distribution of these forms is being contributed from in situ observations made by SCUBA divers.

Ctenophores

These organisms may appear in excessive numbers in plankton tows, especially in temperate estuaries during certain times of the year. Since they tend to break apart and (when dominating samples) somewhat gum up formaldehyde preserved samples, ctenophores should be removed. Their type, number and volume should be recorded and they can be preserved separately. A reference for fixing and preserving ctenophores is listed in the bibliography (Adams et al., 1976).

SECTION 5

SAMPLE PROCESSING

Once they are returned to the laboratory, processing of preserved samples can be initiated. Before proceeding further, large organisms (i.e. medusae, fish larvae, etc.), especially when uncommon to the sample, should be removed. These forms are then counted and identified.

5.1 Subsampling by pipette method

A summary of this method is taken largely from an earlier U. S. Environmental Protection Agency manual (Weber, 1973) and proceeds as follows:

(1) The sample is drained of excess formaldehyde; settled volume is read in a graduated cylinder or Imhoff cone in a volume of water such that plankton makes up 1/5 of the diluted volume.

(2) Sample is stirred with a Stempel pipette; 1 ml of the agitated mixture is withdrawn from the sample.

(3) The subsample is transferred to a gridded culture dish with 5 mm squares.

(4) Pipette is rinsed with distilled water into culture dish, to remove any adherent organisms. Enumerate and identify subsample under a dissecting microscope.

After calculating the dilution factor, the information derived from counting together with estimates of water volume sampled, can be used to derive the total abundance of individual groups or of the total sample. However, considering the relatively small aliquot studied, this method should be used only when rapid "ballpark" numbers are needed. This method, and others employing similar methodology, are not recommended for precise, quantitative analysis of samples.

5.2 Subsampling by splitting

This is the best method for quantitative analysis of zooplankton. Two types of splitters are recommended: the classic Folsom splitter (McEwen et al. 1954) and the newer Burrell et al. (1974) device. The Folsom splitter has received some criticism, due to variations associated with individual handling, but certain simple modifications can alleviate this situation.

Samples should be sorted according to the following guidelines:

(1) Remove excess formaldehyde; place sample in culture dish with 5 mm square grid; place dish under dissecting microscope.

(2) Remove any animals that are rare. Look at numbers within groups that appear in the whole sample. If less than 200 are in any group, remove all individuals within that group. Keyed laboratory counters prove useful for recording individual counts. Animals should be placed in labelled vials of 4% formaldehyde.

(3) Place sample in splitter. Split sample, wash and rinse splitter into sub-splits. Remove and record all individuals of any groups that contain a "workable number" of organisms (100-200 individuals).

(4) Continue splitting and sorting in this manner until an entire sample has been counted.

(5) Splitter should be rinsed with fresh water.

Proper use of a splitter is best illustrated by example:
A 1/2 split contains decapod larvae plus numerous cladocerans and copepods. Decapods are counted and removed until none are left, a total of 150 being counted. Sample is then split and it appears that cladoceran numbers are workable. 175 individuals are counted, removed and recorded. Sample is split again to count copepods, and 156 individuals are counted.

Numbers in total sample:

Decapod larvae	= 150	x 2	= 300
Cladocerans	= 175	x 4	= 700
Copepods	= 156	x 8	= 1248

In situations where certain groups require greater attention or study the "workable number" may be increased. Conversely, in situations where certain groups will receive little study, or when monotypic groups are observed, the "workable number" may be decreased.

Abundance estimates derived from splitting, and information derived from flowmeters, facilitate the calculation of abundance/water volume. Furthermore, fiducial limits can be placed on numbers within groups that have been counted. After samples have been sorted into vials, specialists can determine the species compositions of individual groups.

Other methods for separating organisms by size groups, and density patterns have shown promise for sorting certain individual groups.

5.3 Biomass

5.3.1 Introduction

Within the natural environment, every trophic level contains, at any given time, a fixed amount of living material, composed of several kinds of organisms. This living material is referred to as the standing crop, the biomass being a useful expression of it. The most popular techniques for presenting zooplankton biomass are: (1) Settling volume, (2) Displacement

volume, (3) Wet weight, (4) Dry weight, and (5) Ash-free dry weight. Displacement and settling volume, and wet weight are non-destructive measurements. These methods leave samples intact for further taxonomic and other uses. They are also less time demanding and require less equipment and skill than other methods. They do not, however, provide any insight on the nutritional content of the plankton. If this information is desired, dry weight and ash-free dry weight measurements should be conducted. Ash-free dry weight is considered the most desirable technique for reporting the chemical (organic carbon) composition. Conversion factors between biomass techniques are not always applicable, due to variations in interstitial water content between different sized samples (Wiebe et al. 1975). Smaller samples appear to retain a larger percentage of interstitial water than do larger samples.

5.3.2 Shipboard Treatment

In cases where biomass information is being sought, the use of paired zooplankton samplers is recommended. In this way, a sampler required to undergo biomass analysis is derived from a separate net than is a taxonomic sample. If only a single net is available, subsampling should be conducted rapidly, and with a minimum of damage resulting to the plankton. In cases where organic estimates are required, the sample should be washed and rinsed thoroughly in distilled water. Triple glass distilled water is recommended whenever possible. Samples should be placed in a freezer at -20°C, for storage. If no shipboard freezer is available, maintenance of samples in a well insulated container, filled with dry ice, is adequate.

Microzooplankton samples obtained from water bottles or pumping generally do not provide a great deal of material, therefore wet weight or volume measurements are not recommended. Another problem associated with microzooplankton is that organisms are generally concentrated together with phytoplankton and detrital material. Direct counts and size measurements using a microscope may be a better means for obtaining biomass estimates for microzooplankton.

5.3.3 Settling volume

Settling volume is obtained by allowing plankton to settle in a graduated cylinder or an Imhoff cone. This method is not recommended, since it exhibits wide variations in tests that attempt to reproduce results, and does not compare well with other biomass measures. It is still used by planktologists because of its simplicity and because it leaves material undamaged. This method should be used only in situations where rough approximations of biomass are desired.

5.3.4 Displacement volume

This method measures an equivalent volume of liquid that is displaced by the sample. Displacement volume may be read by several methods of varying complexity. A simple direct measurement proceeds as follows:

(1) Sample is placed in sieve of mesh size equal to or smaller than net used in capture.

(2) Sample is allowed to drain, and transferred to a measured volume of water in a graduated cylinder.

(3) The new volume containing sample + known volume is read.

The displacement volume = new volume minus original measured volume of water.

Another equally valid method reads the volume of water and the interstitial fluid after it has been subtracted from the original (known) volume of sample and fluid.

The more sophisticated Mercury Immersion method provides a better means for removal of interstitial water but requires considerably more time and skill than do direct measurements. Nevertheless, this method has received considerable use by plankton researchers, and several modifications of the original method have been developed (see Beers, 1976). Steps are outlined:

(1) Sample is placed in a chamber with a sintered glass base.

(2) Liquid and interstitial fluid is removed by applying air pressure.

(3) The chamber containing the sample is placed in a dish of mercury, effectively sealing the underside of the glass base.

(4) The chamber is filled to a certain known level by adding water from a burette. The volume of water utilized is read.

(5) After the sample has been removed, the chamber is filled with only water. This volume is read.

Displacement volume = step (5) - step (4), which represents the difference in the volume of water required to fill the chamber, with and without plankton. It is usually expressed in ml/m^3 of water volume sampled.

5.3.5 Wet weight

Wet weight is used to determine the actual weight of the "raw" plankton, once interstitial water has been removed. However, care must be taken to avoid loss of body fluids from the organisms. A disadvantage of wet-weight is the existing degree of variation associated with individual technique. Furthermore in mixed zooplankton samples, individual taxa retain different amounts of water relative to their organic and inorganic constituency. In determining wet weight, the following steps should be followed:

(1) Pre-weigh glass jar.

(2) Strain plankton through plankton gauze of a smaller mesh size than used for capture. Rinse thoroughly with fresh water.

(3) Allow water to drain.

(4) Blot sample on absorbent paper towels until water is no longer absorbed onto the towels.

(5) Transfer sample to glass jar and weigh.

The wet weight is the difference between the weight of the sample + the jar, less the weight of the jar (step 5 - 1). Wet weight is usually expressed in milligrams (or grams)/m^3 of water volume sampled.

5.3.6 Dry weight

Dry weight is a measure of the total plankton weight, after the water content has been removed. To obtain maximum accuracy, drying should be conducted without the loss of any volatile organic material. Freeze-drying (lyophilization) is the best method for extracting water and providing an accurate dry weight value, with minimal biochemical alteration of the sample.

If freeze-drying is not possible, oven-drying at 60°C is recommended. This temperature provides rapid drying and is still low enough to insure a minimum of organic loss. The time required for drying may vary, according to the size of the sample; drying is considered complete when a sample attains a constant weight (two successive readings are not significantly different). Dry weight is usually expressed in milligrams (or gms)/m^3 of water volume sampled.

5.3.7 Ash-free dry weight

Ash content (weight), in samples that were freeze-or oven-dried to constant weight is determined by ashing the sample in a muffle furnace at 500°C. Weights are taken after sample has been allowed to cool. Ash-free dry weight is the measure of organic material in the sample, exclusive of all water and inert organic material. It is calculated as follows:

Ash-free dry weight = dry weight - ash weight. Ash-free weight is usually expressed in milligrams (or gms)/m^3 of water volume sampled.

5.3.8 Large gelatinous forms

Remove from sample and measure separately.

SECTION 6

ADDITIONAL BIOCHEMICAL ANALYSES

6.1 Introduction

In recent years there has been a marked increase in the accumulation of data on the biochemical constituency of marine zooplankton. Procedures for obtaining total protein, lipid, carbohydrate, ash and chitin values were summarized by Raymont et al. (1964), and are outlined below.

Values for the individual components are usually expressed as a percentage of dry weight, obtained from freeze or oven dried samples. Other more detailed types of analyses are mentioned and references to guide potential investigators are offered.

6.2 Total protein

The largest single fraction of most oceanic plankton is usually the protein. The most widely accepted method for determining protein from zooplankton is the colorimetric biuret method.

6.2.1 Biuret method - Procedure:

(1) Homogenize a known dry weight (0-5 mgm) with 1 ml distilled water and 4 ml biuret reagent in a Potter-Elvehjem homogenizer until all purple particles are dissolved.

(2) Filter homogenate through glass paper. Repeat this step until a clear filtrate is obtained.

(3) Transfer to a cuvette and read in a spectrophotometer at 540 mu. The instrument should be zeroed by a blank consisting of 1 ml distilled water and 4 ml biuret reagent.

The optical density (OD) of the sample should be read against a standard curve for bovine albumen. The standard curve should be made up by dissolving a series of known weights (from 0-10 mg) of the albumen in 1 ml distilled water and 4 ml biuret reagent.

6.2.2 Lowry (et al.) method

This is another colorimetric indicator of total protein, and although more sensitive than the biuret method, it is generally considered a micro-method. Its use is recommended in situations where only very small amounts of material are available.

6.3 Total lipid analysis

The lipid is generally the most variable fraction of the marine zooplankton. Constituent classes of this fraction are important as energy reserves and are essential for reproductive and structural components. A common procedure used for calculating total lipid employs extractive and gravimetric methodology.

(1) Lipid is extracted from about 10 mg of dry weight with a 2:1 chloroform-methanol (V/V) mixture in a Potter-Elvehjem homogenizer.

(2) After filtering, the filtrate is washed with a 0.05 N potassium chloride solution. Volume of wash solution added should be approx. 20% of the homogenate volume.

(3) Allow phases to separate by standing (or centrifugation).

(4) Siphon off upper phase.

(5) Transfer lower phase to pre-weighed container and evaporate solvent in a stream of nitrogen.

(6) Only lipid should remain. Record weight and express as a percentage of the initial dry weight.

Modifications of this method using benzene instead of chloroform, and heating samples in a boiling water bath, have been reported to yield accurate results for lipids of individual species. Furthermore, colorimetric procedures also appear adequate.

6.4 Carbohydrate

Carbohydrate has been shown to appear in relatively small amounts in most marine zooplankton. Values are almost always less than 4% of the dry weight. Colorimetric methodology for determination of carbohydrates is recommended. Procedure:

(1) Place a constant dry weight (1-5 mg) in a boiling tube, with 1 ml distilled water, 1 ml 5% phenol solution and 5 ml conc. H_2SO_4.

(2) After allowing to cool for 20 minutes, read optical density (OD) in a spectrophotometer at 490 m μ.

(3) Compare OD's with standard curve for glucose.

6.5 Ash

Obtained from ash-free dry weight analysis (Sec. 5.3.7). Value is material that remains in muffle furnace after sample has been ashed at 500°C.

6.6 Chitin

Crustacean exoskeletons are chitinous, making this material an important fraction to quantify. Procedure is as follows:

(1) A known wet weight (250 mg) is boiled for two hours in 50% NaOH.

(2) Sample is left in solution overnight.

(3) Exoskeletons are removed and washed in dilute HCl.

(4) Exoskeletons are washed in water.

(5) Dry and weigh. This material is ash plus chitin.

(6) Ash in a muffle furnace to burn off any organic material. Only ash should remain.

The chitin value is the difference between the weight of the exoskeleton plus the ash, and only the ash (step 5 - step 6), and can be expressed as a percentage of dry weight. It must be emphasized that the above described procedure is effective only with "wet" or "raw" samples. It does not apply to dried material, since a viscous, gummy solution is produced when the alkaline solution is added. Bamstedt (1974) suggested a modified methodology for estimating total chitin that can be used on dried material. The procedure calls for treatments with 1N HCl in a bath of boiling water, and with 4N NaOH for 20 minutes, also in a water bath. The material is then washed in a succession of fluids. Attempts to reproduce Bamstedt's procedure on dried material in this laboratory (VIMS), proved unsuccessful.

6.7 Other analyses

Amino acid and fatty acid analyses are mentioned briefly but detailed procedures are not presented. References on respective sections should be consulted.

6.7.1 Amino acids

Methodology has been developed for quantification of free amino acids and protein hydrolyzates of certain zooplanktonic crustaceans. Free amino acids are extracted from lipid-free material with hot distilled water. Proteins are precipitated with hot trichloroacetic acid and hydrolyzates are prepared by refluxing with 6N HCl. A column chromatograph and technicon autoanalyzer yield chromatograms for amino acids and protein hydrolyzates.

6.7.2 Fatty acid analysis

Lipid is extracted from the plankton with chloroform-methanol (see Sec. 5.3). Thin layer and column chromatography can be used to separate lipid into principle classes, such as phospholipids, triglycerides, hydrocarbons, wax esters, etc. Gravimetric analysis has, in certain cases, provided relative percentages of the various lipid fractions and studies have assessed the overall significance of these fractions to the organism(s) under examination.

Preparation, hydrogenations and gas liquid chromatography of the methyl esters of constituent fatty acids has provided insight into the fatty acid composition of several species of marine and brackish water plankton.

6.8 Biochemistry of marine zooplankton

A bibliography on this rapidly expanding area of investigation is provided.

SECTION 7

DATA ANALYSIS

After samples have been sorted and other appropriate analyses completed, a cohesive interpretation of data must be developed. Since planktonic organisms are distributed in patches, classical statistical techniques are of limited value. Most zooplankton studies to date, have drawn conclusions from observations of raw data, by ranking abundances of individual species. With expanding computer usage in recent years, techniques such as diversity indices and multivariate analyses, have been developed as tools that determine quantitative relationships among and between zooplankton communities, and between community and environmental parameters.

7.1 Total numbers and frequency of occurrence

Historically this has been the most popular method of presenting zooplankton data. Abundances of individual species (or groups) are listed and ranked. Data on frequency of and periods of occurrence are also calculated. Abundance data are usually expressed in units of individuals per volume of water (m^3, 100 m^3, etc.) sampled. Mean total numbers of individual species (or groups)/ m^3 and percentages of individual species' contribution to the entire sample can also be determined. Confidence limits may be placed on mean numbers of individuals but, unfortunately, due to avoidance and the patchy nature of the plankton, these limits generally cover an extremely wide range. Seasonal trends are usually developed by plotting abundances (no. of ind./m^3) VS. months (or other sampling time period). In addition to baseline and monitoring surveys, studies concerned with day-night differences, spatial variations and ecological zonation of zooplankton communities have employed this type of data interpretation. It is still the most popular, and in many cases, the most meaningful interpretation of data.

7.2 Diversity indices

Diversity indices can be used to establish species diversity gradients in geographical areas. These indices allow for comparisons between community diversity in different areas, with respect to parameters selected (i.e. effects of seasonality, coastal vs. offshore populations of copepods) Diversity indices can also be used as a baseline statistic, in areas where natural or man made changes are anticipated, and can be useful in determining effects of pollutants on changing zooplankton community structure.

These indices are applicable only after the number of species, and the total

number of individuals within a species, have been tabulated. Diversity indices can then offer a statement on the "information content" of each individual in a sample. The Shannon-Wiener H', has been a most widely used diversity statistic and is calculated as follows:

$$H' = - \Sigma p_i \log_2 p_i$$

$$\text{where } p_i = \frac{\text{number of individuals in the ith species}}{\text{total number of individuals}}$$

The index weighs both numbers of species and evenness of species distributions. In systems of low diversity, each successive individual drawn from a sample would, since it is likely to be the same species as a previously drawn individual, contribute rather little to the information of the system; adequate knowledge could be obtained after relatively few drawings. This would result in a low H' value, since individual information input is low. In diverse systems, each successive individual is likely to be different from one previously drawn, and makes a significant contribution to the knowledge of the system. Many drawings would be required to understand the system, and the diversity statistic H' would be high.

Criticisms and modifications in the application of diversity indices have been widespread in the literature. One method claims that the value of H' can be improved by scaling all measurements down to a common sampling size. Other investigators question the value and validity of these indices for ecological research.

Very often, pollution in present day ecosystems results in an overall reduction of certain species and the elimination of others; a few species are, however, capable of exploiting these conditions. Diversity indices may prove useful in pollution-related studies. However, diversity measurements as indicated by H' reflect changes in overall information content rather than enumerate upon an individual species composition changes. In a situation where one species is completely replaced by another, the diversity value would remain constant, yet the change of the species (or several species) may be indicative of significant environment modifications. In cases like this, use of an index of faunal overlap may prove helpful. Of the numerous overlap indices available, a commonly used measurement is the percentage similarity statistic, which ranges from 0 when two samples contain no species in common, to 100 when two samples are identical in both species and abundances.

$$PS = 100 \, (1 - 0.5 \, \Sigma |p_{ia} - p_{ib}|)$$

where p_{ia} = total number of individuals in the ith species in sample a ÷ total number of individuals in sample a.
p_{ib} = the same for sample b.

Thus, diversity indices used in conjunction with either other indices and statistics, or associated observations can, in certain cases, provide an

effective tool in analyzing aspects of zooplankton community structure.

7.3 Multivariate analysis

This type of analysis draws its conclusions by recognizing patterns between variables. As data sets increase in size, the individual's ability to detect trends or patterns naturally declines. The increase in availability and usage of computers has made these analyses feasible and desirable. These techniques condense multidimensional relationships by projecting data onto a reduced number of planes. Thus the multivariate approach tests the reality of a variable, by expressing its variability in terms of an additional coordinate and seeing whether the variable displays a systematic and logical pattern.

Cluster analysis is a multivariate approach that orders sets of taxa on the basis of pre-determined criteria. For example, data from zooplankton surveys may cluster and distinguish groups of samples based upon similarities in composition. The inverse analysis clusters the species according to similarities in their distribution and abundances. Other multivariate approaches, referred to as ordination, make no assumptions in grouping the entities or in drawing boundaries between classes. Recently, principal component analysis has been used to describe and quantify the major elements responsible for fluctuations in zooplankton abundances (Colebrook 1977).

SECTION 8

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